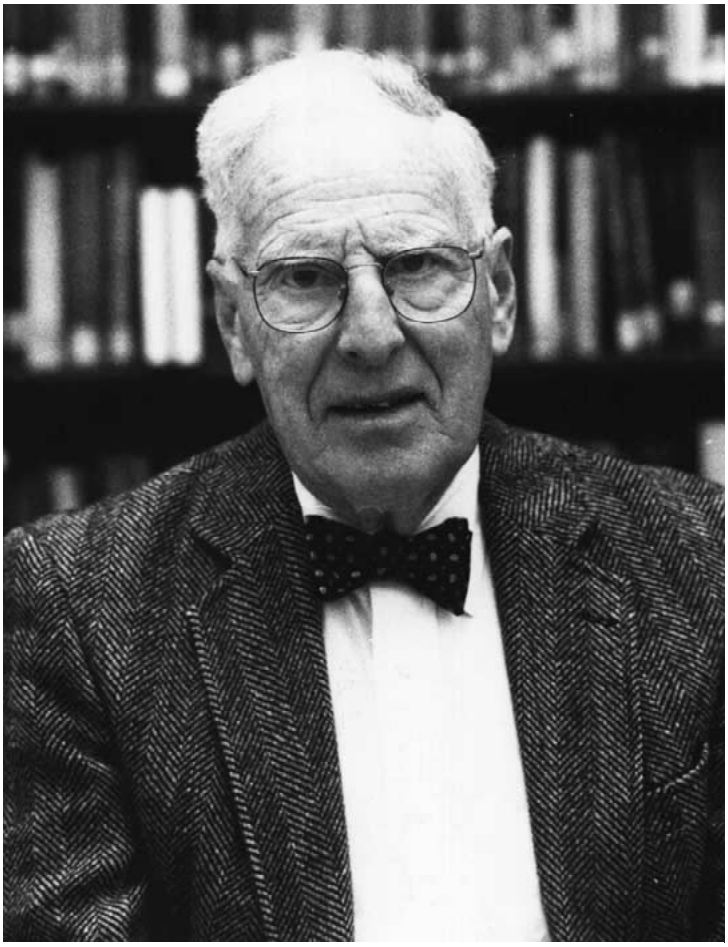

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Personal perspective/Historical corner

Travels in a world of small science*

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Abstract

As a boy, I read Sinclair Lewis's *Arrowsmith* and dreamed of doing research of potential benefit to society. I describe the paths of my scientific career that followed. Several distinguished scientists served as my mentors and I present their profiles. Much of my career was in a small department at a small institution where independent researchers collaborated informally. I describe the unique method of carrying on research there. My curiosity about glycolate metabolism led to unraveling the enzymatic mechanism of the glycolate oxidase reaction and showing the importance of H_2O_2 as a byproduct. I discovered enzymes catalyzing the reduction of glyoxylate and hydroxypyruvate. I found α-hydroxysulfonates were useful competitive inhibitors of glycolate oxidase. In a moment of revelation, I realized that glycolate metabolism was an essential part of photorespiration, a process that lowers net photosynthesis in C_3 plants. I added inhibitors of glycolate oxidase to leaves and showed: (1) glycolate was synthesized only in light as an early product of photosynthetic CO_2 assimilation, (2) the rate of glycolate oxidation consumed a sizable fraction of net photosynthesis in C_3 but not in C_4 plants, and (3) that glycolate metabolism increased greatly at higher temperatures. For a while I studied the control of stomatal opening in leaves, and this led to the finding that potassium ions are a key solute in guard cells. I describe experiments that show that when photorespiration rates are high, as occurs at higher temperatures, genetically increasing leaf catalase activity reduces photorespiration and increases net photosythetic CO_2 assimilation.

Abbreviations: HPMS – α-hydroxy-2-pyridinemethanesulfonate; RuBP – ribulose 1,5-bisphosphate

Philadelphia, Pennsylvania

I was born and grew up in Philadelphia in neighborhoods where I retained my boyhood nickname *Zuni* ('Sonny' in Yiddish). I graduated from Overbrook High School, a large public school with a mixed ethnic population, around my 17th birthday, in June 1941. I was flippant when the yearbook interviewer asked about my career ambition. 'A farmer,' I said thinking that was funny coming from a city boy. Instead the interviewer wrote, "When bigger and better basketball centers come to Overbrook they'll be like *Zuni*." Both prophecies were close. A much bigger and better basketball center did come to Overbrook 15 years later. His name was Wilt Chamberlain, a player who once scored 100 points, which is still a record, in a professional game. The unpublished prophecy about an interest in agriculture was also close.

My father and mother were born in Russia and came to the United States before the First World War and met in Philadelphia. My mother became a registered nurse and survived the influenza (flu) epidemic of 1917 while working in a hospital. My father was apprenticed as a watchmaker at age 13, came to the United States when he was 20, and never attended any school after age 13 until he passed an equivalency

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examination and enrolled in the University of Iowa when he was nearly 30 years old. He then attended the Law School at the University of Pennsylvania, while supporting our family by repairing watches. Upon graduation he sold his tools, and thereafter had an active law practice.

Although we lived in a big city, we were never far from large public parks. When I was not wearing out shoes (the local cobbler told my mother I was a pleasure because I brought him so much work) while playing games in the street, I enjoyed roaming in the park. I was allowed to do this freely. I recall reading the 1925 novel *Arrowsmith* by Sinclair Lewis when I was a teenager. It is a story about an idealistic and stubbornly dedicated research medical microbiologist who worked at an institute patterned after the Rockefeller Institute in New York. The novel made a strong impression on how exciting scientific research could be. I believe this helped kindle my ideal of doing research at an institution dedicated to some public good.

I entered Pennsylvania State College (now University), attracted by a curriculum listed in the catalog called 'Agricultural and Biological Chemistry'. I stayed on that steady course through undergraduate, graduate and post-doctoral work. I then spent 42 years at full-time research at The Connecticut Agricultural Experiment Station. Though 'retired', I continue active research on photosynthesis.

State College, Pennsylvania

Biochemistry or basketball?

I arrived in State College, a small town surrounded by hills near the geographic center of Pennsylvania, for the first time in September 1941. It was not conveniently accessible by public transportation, and its isolation was good for both those who came to study and those who came to play. The coursework for the first 2 years was prescribed, and I soon found friends among serious students and immersed myself in chemistry and botany.

Henry W. Popp gave the first course in general botany. He was a plant physiologist with an interest in light effects on plant growth. I found the subjects covered in plant physiology fascinating. Making drawings of cells as they appeared under a microscope as part of laboratory exercises was less appealing.

I played basketball on the intercollegiate Freshman (first-year students) team they had then, and was on the varsity squad my second year. But the win-at-all-costs attitude soured my enthusiasm and I left the team. One positive aspect of participating in intercollegiate basketball was that because practice took place for several hours every evening, and I was too tired to study afterwards, I was forced to organize my time so that I could keep up with my studies.

My life in State College was so isolated from the outside world that I did not become aware of the bombing of Pearl Harbor on December 7, 1941 until at least a day afterward. It took even longer before I realized what an impact the Second World War would have on my generation. Besides science courses, English composition and a foreign language, there were few opportunities the first years to 'broaden' one's education. Later, in an economics class, I met a young woman named Ruth Goldman to whom I was attracted, and we married in 1945 while I served in the Army and became a tank platoon leader. Shortly after our marriage, I was shipped out first to the Philippines and then to Tokyo, Japan, to be part of the occupation forces. This separation was one of the most painful events of my life.

When I was discharged from the Army in 1946, I still had one semester remaining before graduation. On returning to Penn State after the War, the atmosphere was more focused on studies with a large student population of older veterans anxious to make up for the lost time. I decided to first continue graduate work at Penn State.

I obtained a teaching assistantship in the Department of Agricultural and Biological Chemistry and did research on the amylose and amylopectin content of tobacco leaf starch under the supervision of Carl O. Jensen, the plant biochemist in the Department, to obtain an MS degree. In November, 1947, I wrote letters to biochemistry departments where I thought outstanding research was being done saying I wanted to work for a PhD degree 'on problems in photosynthesis or in related fields of plant chemistry'.

In January 1948, Robert H. Burris from the Department of Biochemistry at the University of Wisconsin offered me a research assistantship to work on biological nitrogen fixation on a Rockefeller Grant shared by him and Perry W. Wilson in the Department of Bacteriology. I looked up the papers by Burris and Wilson and saw how productive they were. The salary of $1100 a year together with the subsidy I had from the government for student ex-veterans, the GI Bill of Rights, would see us through. I accepted the offer enthusiastically and on a hot day in the end of June

1948, Ruth and I arrived by train in Madison to begin our Wisconsin experience.

Madison, Wisconsin

Graduate study with Robert Burris in the home of the 'badgers'

In 1948, the University of Wisconsin was one of the main research centers of nitrogen fixation. Perry W. Wilson, Professor of Bacteriology, had worked on carbohydrate–nitrogen relations in symbiotic nitrogen fixation and had shown that hydrogen was a specific inhibitor of nitrogen fixation by red clover plants. Robert H. Burris was a student of Wilson's and joined the Biochemistry faculty in 1944. In 1948, they collaborated a great deal, and we had joint research conferences every few months.

For many years (1906–1944), Edwin B. Hart (1874–1953) was Chemist 'In Charge' of Biochemistry. Though retired, he came to the laboratory from time to time to look around. We were told that his administrative principle had been, "Get good people and leave them alone". I stored this away as a model.

It took some time for me to get used to the informal midwestern style of calling a professor by his nickname: 'Bob' for Prof. Robert Burris. He and his seven or eight graduate students occupied a large open-space laboratory in a corner of the building closest to some railroad tracks. His office was off the main room and he was always accessible. Occasionally a coal-burning freight train came by towed by a steam-puffing locomotive, and in the summer when the windows were open, the ammonia levels in the laboratory increased enough to raise the blank values in the reagents used to measure it. It was outside this corner of the building that Harry S. Truman came by while riding in a slow-moving open automobile during his successful presidential campaign against Thomas Dewey. Truman sat back and smiled and waved to curious students who came out to see him. There were no Secret Service agents evident and I was close enough to his car to have been able to touch it. I returned to the laboratory while he continued on to give a speech on the university campus.

Research in Bob's laboratory was devoted to different aspects of plant biochemistry, and he was always eager to incorporate new methods to solve problems. He later received many prestigious awards for his accomplishments including the President's National Medal of Science in 1979. His graduate students were working on organic acid metabolism, respiration and carbon aspects of photosynthesis, as well as nitrogen fixation. The use of ^{14}C as a tracer was beginning to be used. Nathan Edward Tolbert (1919–1998) known then as 'Nate' and later as 'Ed' (Figure 1) was in his last year of graduate work studying the enzymatic oxidation of glycolate, a subject that would later lead to his important contributions to understanding the biochemical pathways of photorespiration. Several students associated with Mark A. Stahmann shared the laboratory, working on the biochemistry of plant diseases.

There were probably over 100 graduate students in the department, and I got to know many of them.. I once shared laboratory space in a bacteriology course with a graduate student from the Genetics Department who showed me the new method of replica plating just developed in his laboratory to select antibiotic and nutritional mutants rapidly in bacteria. There was a sense of the excitement of rapid developments in biochemical research in all fields and it gave hope that one might participate in it.

Most of the graduate students had been in the military service during the War and were older and more focused. Only a few students spent time at the Badger Tavern, the beer joint across the street from the Biochemistry Department. Many of us were married and some had children. We were in a hurry to get ahead, and this eagerness fit in well with the professors who were in a hurry to use new tools to answer important scientific questions. It was a good environment for learning and working.

However, my first summer in Madison did not start well. Bob was away when I arrived and the older students counseled me to take the enzyme course that was given during the summers by Marvin J. Johnson and Henry A. Lardy. Johnson, an energetic and fast-talking professor, gave most of the lectures in the beginning. Once a week he would give a written exam (called quiz these days) that had to be turned in after exactly 5 minutes. It usually consisted of one question, a Johnson original requiring the solution of a tricky problem often involving enzyme kinetics. I was frequently unable to solve these problems in the allotted 5 minutes, and this troubled me. Yet, some of the graduate students who did well in the 5-minute quizzes never did outstanding research after they left graduate school. I think the reason may be that those successful in research must be adept at sorting out weak signals from a strong background of noise. To do this well requires

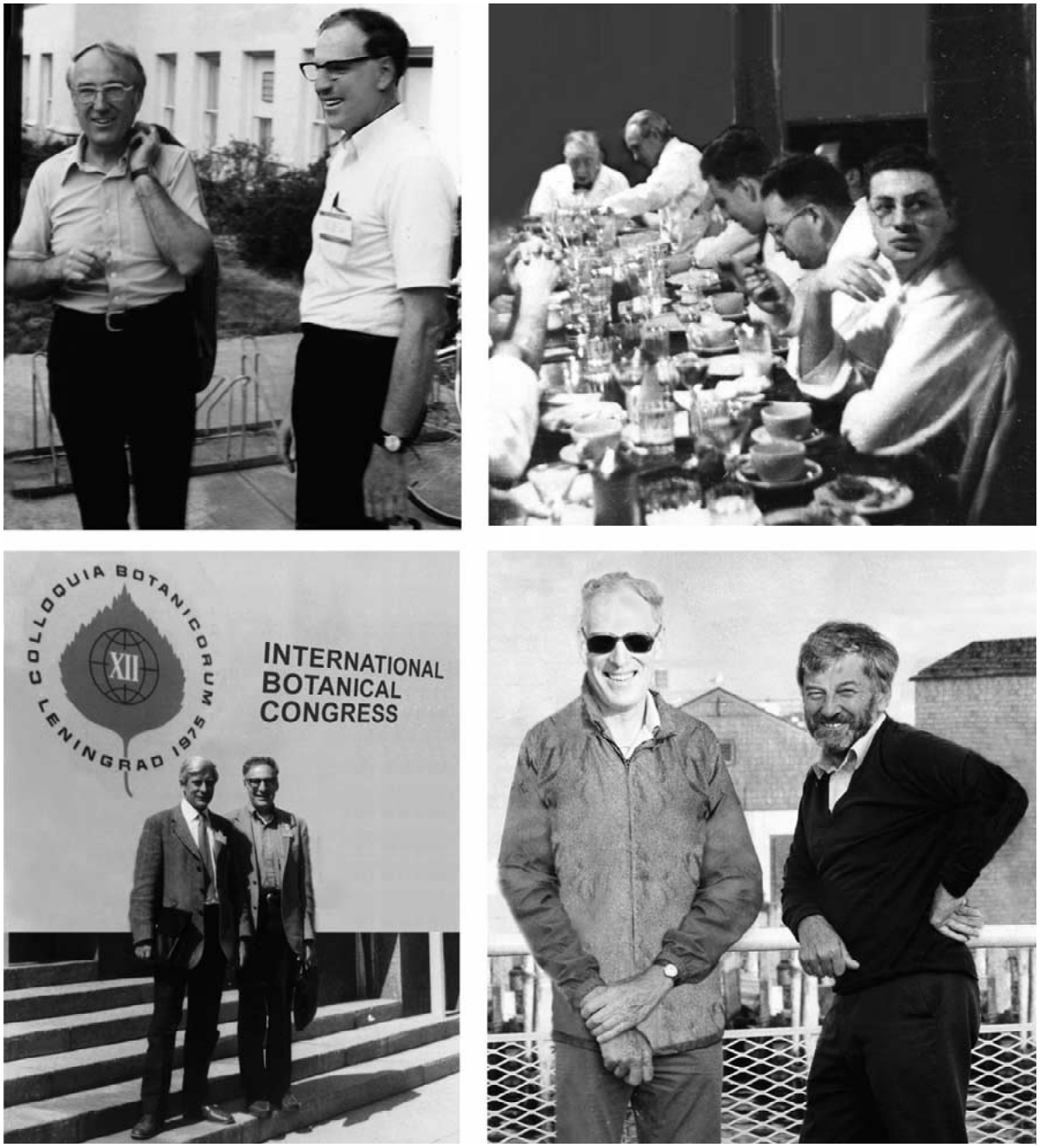

Figure 1. Top left: Nathan E. Tolbert and the author at a meeting of the American Society of Plant Physiologists, Corvallis, Oregon, August, 1975. *Top right*: Lunch at a festive occasion at New York University School of Medicine, May, 1952. From rear to front:Otto Loewi, Severo Ochoa, the author, Efraim Racker and Seymour Kaufman. Lower*left*: Vernon Butt and the author, 1975. *Lower right*: The author and David A. Walker, Block Island Ferry, July, 1982.

knowledge, deep thinking and persistence, and not necessarily being the first one off the mark in a race.

Is ammonia the first stable intermediate of nitrogen fixation?

The only mass spectrometer available for measuring ^{15}N isotope ratios was in the Chemistry Department. It had been built by a graduate student who had already left. Later, the instrument was relocated in the Biochemistry Department, and because of Bob's skill and diligence in finding and repairing leaks in the glass tubing in the vacuum system, it was on occasion possible to do ^{15}N determinations.

The big controversy in biological nitrogen fixation at the time was whether or not ammonia was the first product. A departed graduate student had supplied ^{15}N-nitrogen to soybean root nodules and Bob asked me to fractionate the protein hydrolysate to determine the distribution in different amino acid fractions. Classical methods used involved the precipitation and separation of groups of amino acids according to their chemical properties with different reagents. To learn this procedure, I practiced on an acid hydrolysate of the milk protein casein, and when I had mastered the methods I repeated the fractionation on the experimental material from soybean root nodules that had been left behind. The mass spectrometer was not working for much of the time, and when I finally assayed the fractions for ^{15}N there was little enrichment above background in any amino acid. We discovered that there had been a leak in the flask containing the ^{15}N-nitrogen; thus, the experiment unknowingly had been conducted without the use of enriched ^{15}N. These failures took up my first 6 months in Madison.

Fortunately, at just about this time, the first methods for separating amino acids by column chromatography were being described. These methods required little time to learn. A while later, a reliable commercial isotope ratio mass spectrometer (Consolidated-Nier) was given to the laboratory by a benefactor of the University. Thereafter, my work went smoothly enough for me to finish my work for the PhD degree 2.5 years after arriving in Madison. I did repeat the experiment with soybean root nodules and found that glutamic acid had the highest ^{15}N specific activity, consistent with ammonia being the first intermediate in nitrogen fixation (Zelitch et al. 1952).

An important serendipitous experiment I participated in occurred because Eugene D. Rosenblum, a student working with Wilson, was investigating nitrogen fixation in the anaerobic organism *Clostridium pasteurianum*. He made the surprising observation that up to fifty percent of the total nitrogen fixed could be excreted into the medium. Since I was working with ^{15}N-nitrogen, we gave growing cells highly labeled-nitrogen and assays revealed that the ^{15}N-ammonia in the supernatant solution had nearly half the specific activity of the nitrogen we supplied (Zelitch et al. 1951). The fortuitous circumstance that the medium used had allowed the excretion of ammonia faster than it could be converted into amino acids provided a direct and unequivocal demonstration that ammonia was a key intermediate in nitrogen fixation.

In the fall of 1950, I talked with Bob about my future once I finished my work in his laboratory. The exciting discoveries in biochemistry at the time were being made in laboratories studying reactions and reaction sequences with purified enzymes and the testing of new ideas about enzyme regulation. Severo Ochoa (1905–1993), at New York University College of Medicine in New York City, one of the international leaders in this field was scheduled to give a lecture in Madison in a few weeks. Bob suggested Ochoa would be a good person to hook up with.

First meeting with Severo Ochoa

I wrote to Ochoa and several weeks later Bob and I met with him in Bob's office. After a brief conversation, Ochoa said that I could join his laboratory on July 1 of the next year. He was interested in continuing the work begun in Burris's laboratory on the oxidation of glycolate and suggested I do further research on that problem with him. Burris approved of the idea, and he left saying that we should correspond further about the details and he would support my applications for postdoctoral fellowships to support my stay for 1 year.

Ochoa had very broad interests in carboxylating and decarboxylating enzymes. The malic enzyme, that converts malate into pyruvate and CO_2 in a readily reversible manner, had been discovered in his laboratory (Ochoa et al. 1948). His group was in the thick of races with other biochemists to understand the mechanisms of the oxidation of pyruvate and α-ketoglutarate in the citric acid cycle. Wolf Vishniac and he had shown (Vishniac and Ochoa 1952) that illuminated chloroplast preparations could reduce pyridine nucleotides and they understood that this could provide the reducing power used in photosynthesis for converting CO_2 to carbohydrate.

By the spring of 1951, I had received a postdoctoral fellowship from the National Research Council that would allow us to live in New York and work with Ochoa. I was very excited about becoming a colleague of such a distinguished company of research scientists, and this feeling was reinforced after I had met his group while visiting back East in December, 1950.

New York City, New York

Life in the fast lane with Severo Ochoa

Severo (Figure 1) was Professor of Pharmacology and Chairman of the Department when I arrived there. He would become a Nobel Laureate in 1959. There were about five staff members who were biochemists. Three Assistant Professors (Seymour Korkes, Joseph R. Stern and Seymour Kaufman) did almost full time biochemical research with Severo, and one senior person, Sarah Ratner, worked independently in her own laboratory on amino acid biochemistry. The staff members each gave one or two lectures a year to medical students taking a course in pharmacology. There was, in fact, one bona fide pharmacologist in the Department who kept to himself and rarely interacted with the rest of the Department. Severo also supervised four or five postdoctoral students, several established visiting scientists, and one graduate student. Otto Loewi (Figure 1), 1873–1961, a Nobel Laureate in 1921 when he was in Vienna, was an Emeritus Professor. He came by occasionally and loved to reminisce with willing young listeners like myself about interesting people he had known in Vienna including Sigmund Freud. He told me the story of how he conceived the nature of chemical transmission in the nervous system during a sleepless night. He woke up and went to the lab and did the experiment on a frog nerve by morning for which he later got the Nobel Prize.

Severo was tall, thin and distinguished looking with white hair, and people rightly said that he looked much like the figure in El Greco's oil painting 'Portrait of a Cardinal' that is displayed at the Metropolitan Museum of Art in New York. He was born in Spain, spoke English with a musical Spanish accent, and did research work with famous biochemists in Germany and in England before coming to the United States of America. His Department was housed on the top floor of an old building, since torn down, on the corner of First Avenue and 26th Street that also housed the New York University School of Dentistry. To get to the laboratories on the top floor, one took a slow-moving elevator, walked past the anatomy labs of the Dental School and its cadavers and the smell of formaldehyde, into an oasis of active research at the rear of the same floor.

The method of doing research in Severo's Department was unusual and deserves description. The strong Professor system that still existed in Europe at that time, but not in the United States, was in force here. The Department working space consisted of about half a dozen small labs on both sides of a corridor and several cold rooms. I shared a narrow laboratory room with Wolf Vishniac, then starting his second year of postdoctoral work. He was still working with isolated chloroplasts.

The windows of our laboratory looked out over the rooftops of tenement houses, and on one roof a man kept homing pigeons that he would allow to exercise so daydreaming scientists could occasionally watch them soaring in large circles. Sometime during each morning, Severo would come by to inquire about the work being undertaken, to see results, ask a question or make suggestions.

Severo was generous in sharing personal research experiences and insights with me. For a long time, he told me he had worked unsuccessfully to understand the mechanism of α-ketoglutarate oxidation in the citric acid cycle. Finally, he dropped the problem. Then, when the discovery of coenzyme-A was announced, he realized that it could be the missing link in the oxidation, and Seymour Kaufman (Figure 1) showed that succinyl-CoA was an intermediate in the reaction (Kaufman et al. 1953). "To skim the cream off a problem it must be tackled when the time is ripe", he told me. I often remembered this dictum and wished I were better at balancing it with the stubbornness required to solve difficult problems. In retrospect, I think my tenacity made it difficult to give up soon enough and admit that problems I worked on were not yet 'ripe'.

He had a similar routine of visiting all of the Assistant Professors in the Department every day as he had for me. I thought this an ideal way to learn and expand my knowledge as an apprentice with a master, but the more experienced researchers chafed at what they considered an intrusion into their intellectual domain and could not wait to become independent researchers. Almost all left for academic positions elsewhere in the next year in order to do this.

Lunch was taken in a cafeteria on the ground floor of the building at a table long enough to ac-

commodate all the scientists who were free at that time. Efraim Racker (Figure 1), whose laboratory was several floors below ours in the Microbiology Department, joined the group regularly. When everyone was seated, Severo would start the day's discussion by saying something like, "I just read a paper by so-and-so about such-and-such in which it is claimed that . . . What do you think?" Thereupon the strengths and weaknesses of the paper were thoroughly debated. Severo had a phenomenal ability to recall details in papers he had read, and retrieved this stored information rapidly. Sometimes someone would describe an interesting or puzzling result in their experiments and people would make comments. During the year, many prominent visiting biochemists from Europe and elsewhere would stop by during our lunches and share news about work in their laboratories or gossip about who was rumored to get the Nobel Prize. All this added to the feeling of being at the forefront of worldwide research discoveries. Lunchtime with Severo was much more than cafeteria food. It was a rigorous daily seminar on enzyme chemistry and biochemical mechanisms.

Although Severo had outside cultural interests, during the whole year I never heard a non-scientific subject discussed at lunch except one time when he mentioned in passing that he had been to the opera the night before. The laboratory was open six days a week, but Saturdays were short days. Early in the afternoon, Severo and the whole Department would go out for lunch, usually at some nearby ethnic restaurant such as The Balkan. The week's laboratory work ended this way in a congenial atmosphere. After lunch, I would hurry home to spend some time with our two young children.

There were no facilities for growing plants in the Department, so I used store bought spinach leaves to purify glycolate oxidase. A large vegetable market that spilled out into the sidewalk was conveniently located a short distance from the laboratory. They sold fresh spinach year round, and whenever I needed leaves I would purchase some spinach on my way to work in the morning. After a while, the market owner recognized me as the frequent buyer of a single item, spinach. When I told him I worked at the Medical School and was grinding up the leaves, he nodded and said, "I always knew spinach was good for making medicines for keeping people healthy."

Getting to know enzymes oxidizing glycolate and reducing glyoxylate

Benson and Calvin (1950), based on short-time labeling experiments with $^{14}CO_2$, reported that glycolic acid arises as an early photosynthetic intermediate. In retrospect, I can say with confidence that they lost some glycolic acid on their paper chromatograms because free glycolic acid is somewhat volatile and the separations were carried out using acidic solvents. It would take until 1971 for William Ogren's laboratory to show that glycolic acid is derived from phosphoglycolic acid produced by the oxygenase reaction associated with RuBP carboxylase, the enzyme responsible for CO_2 fixation in photosynthesis. The important role of glycolic acid metabolism in photorespiration was not clarified until the 1960s.

Clagett et al. (1949) partially purified the glycolate oxidase from tobacco leaves and found the oxidization of glycolic acid proceeded through glyoxylic acid to formic acid and CO_2. In New York, I purified glycolate oxidase some 500-fold from spinach leaves and showed that riboflavin phosphate was the prosthetic group. The purified flavoprotein catalyzed the oxidation of glycolate to glyoxylate and hydrogen peroxide, but did not oxidize glyoxylate. In the absence of catalase, the hydrogen peroxide reacted rapidly and non-enzymatically with glyoxylate to yield formate and CO_2. With excess catalase present, the hydrogen peroxide was destroyed and the final product was glyoxylate (Zelitch and Ochoa 1953); the sequence was also described by researchers in England (Kenten and Mann 1952) as follows:

(1) Glycolate + O_2 → glyoxylate + H_2O_2 (glycolate oxidase)
(2) H_2O_2 → H_2O + $1/2O_2$ (catalase)
(3) Glyoxylate + H_2O_2 → formate + CO_2 + H_2O (peroxidation)
(4) Sum (1) + (2): glycolate + $1/2O_2$ → glyoxylate + H_2O
(5) Sum (1) + (3): glycolate + O_2 → formate + CO_2 + H_2O

In the spring of 1952, Severo was to leave for over a month's visit to Europe just about the time I had finished my work on glycolate oxidase. He proposed that during his absence I look for enzyme systems in leaf extracts that might synthesize glyoxylate by a direct carboxylation reaction.

Because it was easier to look for an enzymatic oxidative decarboxylation, I added glyoxylate to crude homogenates of spinach leaves to see if oxygen up-

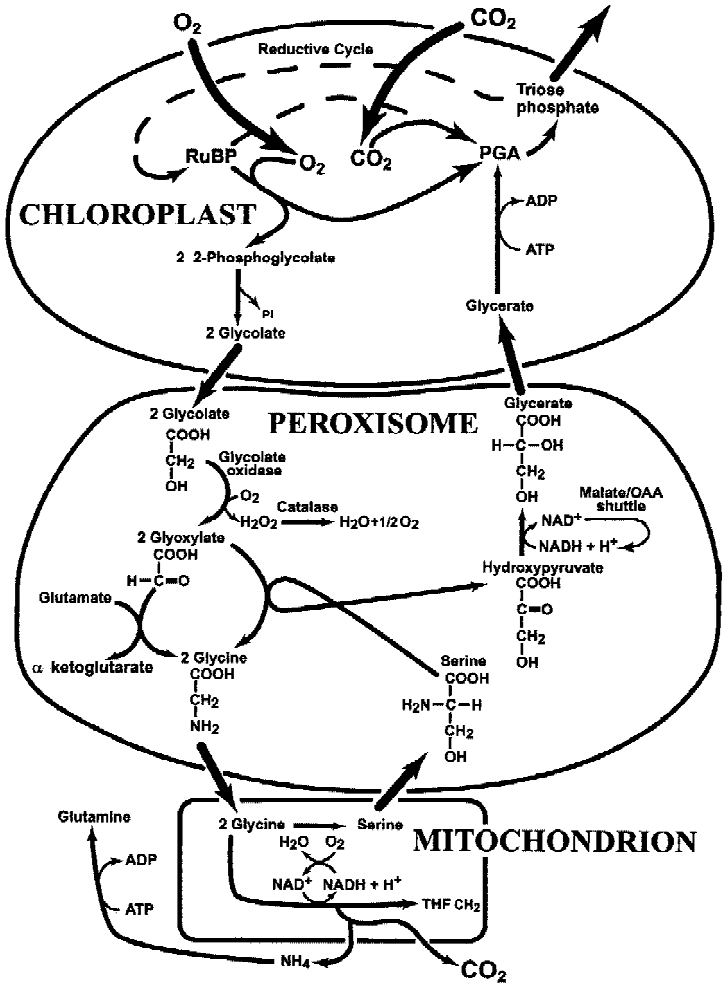

Figure 2. The relation between photosynthetic CO_2 assimilation and the cyclic photorespiratory pathway when catalase activity is present in excess. The uptake of CO_2 and O_2 in chloroplasts produces phosphoglyceric acid (PGA) and 2-phosphoglycolate from which glycolate arises. The glycolate is oxidized and produces CO_2 during the decarboxylation of two glycines to produce serine (1 CO_2 from 4 C yields a stoichiometry of 25%). Phosphoglyceric acid (PGA) is regenerated when the photorespiratory cycle is completed making it a futile cycle. Under some conditions, when catalase activity is limiting, additional CO_2may be produced from the nonenzymatic peroxidation of glyoxylate and hydroxypyruvate in the photorespiratory pathway.

take was stimulated. To my surprise, oxygen uptake by the leaf preparations increased greatly upon the addition of glyoxylate. I soon learned that the increased oxygen consumption was not due to the oxidative decarboxylation of glyoxylate, because the glyoxylate concentration was unchanged at the end of the experiment. The oxygen consumption on a molar basis was also greater than the amount of glyoxylate added, so it had a catalytic effect on oxygen uptake. After considerable anguish about these puzzling results, I finally surmised that the homogenates must contain endogenous substrates that were being oxidized and a new enzyme, perhaps a pyridine nucleotide-linked glyoxylate reductase, was present that shuttled glyoxylate back to glycolate. The glycolate oxidase reaction thereupon took up oxygen and produced glyoxylate again. This shuttling process then oxidized substrates in the homogenate that were linked to enzymes able to produce reduced nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP).

I then partially purifed the glyoxylate reductase I had discovered, which could function with NADH and NADPH in such preparations. The new enzyme would not reduce pyruvate (hence it was not a lactic dehydrogenase). I was able to reconstruct the shuttling

process between glycolate and glyoxylate with purified enzymes, and by the time Severo returned from abroad these experiments were almost finished. He was pleased with my results and took an active part in editing the paper (Zelitch 1953), but insisted his name not be included as an author because the work was done while he was away without his input. I was impressed by his ethical behavior and believe it may have been the only paper published from his group in which he was not a co-author.

Later, I isolated a crystalline glyoxylate reductase from tobacco leaves that was specific for NADH (Zelitch 1955), and showed that the enzyme also catalyzed the reduction of hydroxypyruvate to D-glycerate. Stafford et al. (1954) independently showed that leaf extracts had hydroxypyruvate reductase activity. It is now known (Givan and Kleczkowski 1992) that these were the same enzyme and that hydroxypyruvate is the preferred substrate. It is called peroxisomal hydroxypyruvate reductase-1, and catalyzes an essential step in the photorespiratory carbon cycle (Figure 2).

Antonio M. Gotto, an American Rhodes Scholar, and I partly purified a glyoxylate reductase specific for NADPH in work which began while I was on sabbatical leave in Oxford, England (Zelitch and Gotto 1962). It is a cytosolic enzyme and has a strong preference for glyoxylate as a substrate (Givan and Kleczkowski 1992). This enzyme may function to minimize the chance that any glyoxylate escaping transamination to glycine in the photorespiratory pathway might enter the chloroplasts where it might inhibit photosynthesis.

A position opens up for "someone to remain for a considerable time"

In July 1951, soon after starting work in New York, I had word from Bob Burris in Madison telling me there was a vacancy in Hubert B. Vickery's laboratory in the Biochemistry Department at The Connecticut Agricultural Experiment Station in New Haven. Bob wrote that the salary was $4500 "which is not princely for these days". A week later, Vickery wrote he had a position for someone "to act as my assistant in the current research work of the laboratory". I had read many of Vickery's papers on the organic acid metabolism of leaves, and had a high regard for them especially a long series of papers by G.W. Pucher and Vickery.

Since I wanted an opportunity to try my hand at independent research, the offer to be under Vickery's thumb as his assistant was not attractive. But the idea of working in New Haven at an institution with a splendid reputation devoted to solving problems of importance to citizens was in keeping with my boyhood *Arrowsmith*-like ideal. I had an interview with Vickery in New Haven and he assured me he wanted someone to do just the kind of work with enzymes I was interested in, and that I would be able to set the direction and goals. There was only one snag: He wanted someone to start right away. I told him I would not give up the opportunity and experience I was getting with Severo in New York, but that I would want the position if it were available next June. The interview ended on this uncertainty. All the negotiations and interviews were with Vickery and no search committees or administrators took part.

By November of 1951, I was able to send reprints of my papers on nitrogen fixation from Wisconsin to Vickery. He wrote that he was "looking for a person, preferably someone who would wish to remain with us for a considerable period of time". I confirmed my interest in the position. Late in November, he answered that he was unable to find someone "with the training I need who is available by the first of the year" and agreed to keep the position open for me.

In the spring of 1952, I visited New Haven again to discuss equipment needs and met James G. Horsfall, the Experiment Station's Director, for the first time and for only several minutes. He said he hoped I would enjoy working at the Experiment Station, and on departing he said, "You should know we are not gravel counters here." I was unsure what that meant and merely nodded. Only later did I realize that it was his way of telling me that trivial work was not tolerated. I liked that. Thus fortified by good experience and high standards observed in graduate and postdoctoral work, I began research on June 23, 1952 in the laboratory where I remain to this day.

New Haven, Connecticut

The first agricultural experiment station in the nation

The institution where I work is like no other that I know. It deserves some background explanation and description. Samuel W. Johnson (1830–1909), a wealthy farmer's son in New York State, came to Yale University in New Haven to study chemistry and became professor of agricultural chemistry at Yale in 1855. In 1853, he had gone to Germany to study and near Munich discovered a 'Versuchsstation' with

chemists on its staff "devoted to the advancement of agriculture by scientific investigation carried on in close connection with practical experiment". This model stimulated him to try to establish such an institution in New Haven and he worked hard to do this by giving numerous speeches and writing articles about the benefits such an institution could provide. Finally, in 1875, the Connecticut Legislature created 'The Connecticut Agricultural Experiment Station' locating it at first at Wesleyan University in Middletown, and in 1877 it was moved to New Haven and Johnson was appointed Director.

As Director, Johnson continued to be an advocate for basic research as much as applied. The Station was located near Yale University to be close to an 'academy'. In 1888, Thomas B. Osborne (1859–1929), another Yale graduate, was appointed to create the Biochemistry Department. He became world famous as a protein chemist (his method of separating albumins and globulins is still cited) while studying the properties of crystalline seed proteins. Upon feeding animals pure proteins as a source of nitrogen and showing they were inadequate, he and a Yale professor, Lafayette B. Mendel, described the first essential amino acid requirements in animal nutrition. Osborne also discovered vitamin A in butterfat and published his findings three weeks after Elmer C. McCollum's paper appeared in the same journal.

Hubert B. Vickery (1893–1978), a Canadian who also did his graduate work in the Chemistry Department at Yale, was appointed to the staff in 1922 and became Head of the Biochemistry Department on Osborne's retirement in 1928 (see Zelitch 1985 for a biography of Vickery).

The Experiment Station was and still is small. When I arrived, there were about eight departments with academic names related to chemistry, plant sciences, soil and insects with a total appointed scientific professional staff of about 40. In the intervening years the departments have been reorganized in several instances. Department of Biochemistry became Department of Biochemistry and Genetics, for example, but the total staff size has not increased. Operating and capital funds come mostly from the State legislature, and are augmented by the so-called Hatch Funds that are apportioned to the states by the Federal government for agricultural research.

I did full-time research. The nearness of Yale University, where some scientists had large groups more representative of big science, allowed me to attend seminars and make other professional contacts. In 1960, Joseph S. Fruton invited me to become an adjunct faculty member in the Biochemistry Department, and later I also became affiliated with the Biology Department. On an irregular basis I gave a graduate course a number of times sponsored by both Departments called 'Perspectives in Photosynthesis'.

Collaboration with independent scientists within a department and with those of other departments at the Experiment Station was easy if the interests and skills meshed, and I have published joint papers with colleagues in this and several other departments. For more than a year I spent part of every day as a guest in Timothy Nelson's laboratory half a mile down the street in the Biology Department at Yale while still doing work at the Experiment Station. At Yale, I wanted to learn how to clone DNA in order to make transgenic plants that overproduce and underproduce leaf catalase. Brian McGonigle, a graduate student in Nelson's laboratory who was very helpful during my visit there, once said, referring to my travels back and forth on Prospect Street, that I would be smart to apply for frequent flier status. The work I started at Yale was then continued and completed at the Experiment Station.

Throughout my career I have done my own work in the laboratory every day, even later when I had administrative responsibilities. I enjoy working in the laboratory and believe that while performing experiments, insights may be revealed and ideas may arise that might otherwise not be brought to light.

I learned to appreciate that for such a system of small science to work successfully, leadership and management skills are needed at the top. James G. Horsfall, an eminent plant pathologist who pioneered the use of organic fungicides, was Director when I arrived in1952 and led the Experiment Station until 1971. He was born in Arkansas, and though he spoke with a Southern drawl, he was as successful in gaining the confidence of our Yankee State Legislature as he was in promoting his vision of the Station to scientists and citizens alike. He preached that "good science is good for the citizens" who support us and the Legislature seemed proud that they were sustaining an institution with high scientific standards that could also anticipate and "put out fires" when the need arose.

After I had been at the Experiment Station about 7 years, Horsfall said I should take a 6-month sabbatical leave in association with an outstanding biochemist in Europe because it would be valuable and broaden my experience. As a result, I contacted Hans Krebs in the Biochemistry Department, Oxford University and

thanks to some help from a Guggenheim Fellowship, I was associated with his group in 1960 while working a few steps away in Vernon Butt's (Figure 1) laboratory in the Botany Department. My location was ideal, and contacts with Vernon and others there helped enlarge my professional horizons. My family was fortunate to find a 600-year old house in which to live, Chaucer's Cottage, in the village of Woodstock eight miles from the University. Our house was close by the entrance gate to Blenheim Palace with its great park and landscape architecture still intact as it was designed 300 years earlier by Capability Brown. Our three children, Helen, Bernard and Deborah, attended the local Council school and picked up English accents after a few days. The visit created a number of valuable professional and personal friendships. Horsfall was right.

On another occasion, Horsfall suggested it would be good for me to write a book, especially a good one. He said publishing scientific papers is important, but even scientists retain the faith expressed by children when they first learn to read, namely that if something appears in a book it must be right. That nudge led me to spend almost every evening during one exhilarating year completing the book *Photosynthesis, Photorespiration, and Plant Productivity* (Zelitch 1971). The feedback of this effort showed once again that Horsfall knew what he was talking about. One of the most moving compliments I received about the book (though without royalties) came after a lecture I once gave at the University in Piracicaba, Brazil. A student approached me after the talk and said she had traveled 200 miles to hear me and asked if I would autograph her copy of my book, which turned out to be a complete photocopy. I gladly signed it.

What does glycolate metabolism do in leaves?

After a seminar I gave at Yale, a scientist, C.W. Partridge, pointed out to me that sulfanilamides were inhibitors of the metabolism of *p*-aminobenzoic acid, and perhaps α-hydroxysulfonic acids by analogy might be effective in inhibiting the metabolism of glycolate, an α-hydroxycarboxylic acid. I examined my organic chemistry texts and was reminded that aldehdye–bisulfite addition compounds are in fact salts of α-hydroxysulfonates. These are stable compounds at near neutral pH values. I prepared a number of these compounds and found all were effective competitive inhibitors of glycolate oxidase. Even sodium bisulfite alone was a good inhibitor because it produced glyoxylate–bisulfite (disodium sulfoglycolate, NaOOC-CH(OH)-SO_3Na) in the course of the reaction (Zelitch 1957).

I thought that if the glycolate oxidase reaction were inhibited in leaves, glycolic acid should accumulate at the rate at which it is synthesized if synthesis is not inhibited. When excised tobacco leaves were placed in solutions of α-hydroxysulfonates or sodium bisulfite in the light, glycolic acid accumulated 10-fold in the light in the first experiments. After 1 hour, if I put the leaves in the dark, the glycolic acid levels fell to its normal level. Then, if the leaves were placed back in light once more, glycolic acid accumulated as before, confirming that glycolate was synthesized only during photosynthesis (Zelitch 1958).

I next found that HPMS (α-hydroxy-2-pyridinemethanesulfonate) was more effective in penetrating tobacco leaf cells than other analogous glycolate oxidase inhibitors, and experiments with this inhibitor showed that irradiance levels similar to those needed for maximal photosynthesis was required for optimal glycolate synthesis. When excised leaves with their bases in inhibitor solution were given $^{14}CO_2$ in high light, as much as 50% of the carbon fixed accumulated in glycolic acid. I concluded that in the normal leaf in sunlight, glycolic acid has a "a central position... in the process of respiration and photosynthesis in leaves" (Zelitch 1959).

A side trip in studies on stomatal control

The CO_2 entering leaves during photosynthesis and the H_2O leaving during transpiration must diffuse through leaf stomata that function as resistors. I noticed one day while doing experiments with excised leaves in the greenhouse on a hot day in bright sunlight that leaves standing in water wilted while leaves in α-hydroxysulfonates solutions remained fully turgid. I realized that the stomata were probably being closed by the inhibitor, and did simple measurements that confirmed transpiration was decreased by the inhibitor solutions. Since I was not then able to measure stomatal apertures easily, I did nothing further with this observation for a while.

Then, when I was working at Oxford University, a technician showed me a technique she was using to make impressions of leaf surfaces for electron microscopy using silicone rubber preparations that smelled of mint. It had this odour because it had been bought from a dental supply company and was usually sold to dentists for making dental impressions. When I

returned home, I contacted the Silicone Products Department at the General Electric Company and they sent me odorless samples to try out. I found that a viscous fluid silicone rubber (RTV-11) when well stirred and mixed with a catalyst could be gently spread with a spatula over an area of leaf or leaf disk. Enough catalyst was used so the fluid was converted into a firm piece of rubber within about 2 minutes. I then removed the rubber impression and painted them with a thin film of colourless nail polish diluted with acetone. Stomatal apertures were measured on the dried film, a 'positive', under the microscope. This method allowed large numbers of samples to be collected at one time and provided a permanent record without damaging the leaf. In my first experiments, I reported that in light HPMS supplied through the base of excised leaves closed stomata and reduced transpiration. Since the CO_2 taken up in photosynthesis must first pass through the stomata and also diffuse through an aqueous phase before reaching the chloroplast, closing stomata should affect a greater proportion of total diffusion in the case of transpiration and have a smaller effect on photosynthesis. I did observe that when stomata were closed by HPMS on leaves, transpiration usually decreased with a smaller effect on net photosynthetic carbon assimilation (Zelitch 1961).

I later found a published paper that said that phenylmercuric chloride sprays decreased transpiration when used as a fungicide on tomato. This led me to try dilute solutions of phenylmercuric acetate, which is more water soluble, and this compound closed stomata when sprayed on leaves only on the surface sprayed, and closure persisted for about 14 days. I did a number of experiments using this inhibitor in collaboration with Paul E. Waggoner, a climatologist at the Experiment Station. We compared transpiration rates and uptake of tracer quantities of $^{14}CO_2$ in treated and untreated excised tobacco leaves in a well-stirred chamber in the light. In 9 of 11 experiments, induced closure of stomata reduced transpiration relatively more than carbon assimilation (Zelitch and Waggoner 1962a), and closing stomata of tobacco plants in a greenhouse consistently diminished transpiration (Zelitch and Waggoner 1962b).

One of the lasting friendships we established during our stay in England was with David A. Walker (Figure 1), then working in London, and his family. In 1962, he and his family spent 6 months visiting in New Haven, and David and I worked together on the biochemical mechanism of stomatal opening supported by a grant from the Charles F. Kettering Foundation. He has already described the quaint nature of our laboratory with warmth and good humor in *Photosynthesis Research* (Walker 1997). His skills in the laboratory and creative mind made working with him a pleasure. Back then, no one would have been surprised at the important advances he would later make to the understanding of the enzymatic regulation of the photosynthetic carbon cycle in isolated chloroplasts and leaves. In experiments with leaf disks we showed that a number of metabolic inhibitors can prevent stomatal opening or cause open stomata to close, that stomatal opening increased with temperature, and that external oxygen was required for opening (Walker and Zelitch 1963).

Some 7 years later, after I had turned my attention back to glycolate metabolism, I attended a lunch seminar given by Brij L. Sawhney, a soil scientist at the Experiment Station. He was studying the binding of cations by soil minerals using an electron microprobe to accurately determine the concentration of elements like potassium in a 1–2 μm diameter region based on the element's characteristic X-radiation. There had been suggestions in the literature that an increase in the potassium concentration in the guard cells of stomata could provide some of the essential solute increase to bring about stomatal opening. We decided to test this. I performed the experiment and provided specimens of leaf epidermis with stomata at different stages of opening, and Brij did the electron microprobe determinations. We found a linear increase in potassium ion concentration up to 0.29 M in guard cells as stomata opened up fully. This suggested potassium as an important solute in increasing the osmotic potential of these cells (Sawney and Zelitch 1969). These collaborative experiments were conceived and carried out in less than 1 month, and an added pleasure was that our paper was included in a list of most highly cited papers.

Photorespiration involves glycolate synthesis and oxidation

Eugene Rabinowitch in his comprehensive treatise on photosynthesis considered the possibility of an accelerated respiration in the light and cited apparently contradictory results. Then he wrote, "The possibility of such an effect is a nightmare oppressing all who are concerned with the exact measurement of [gross] photosynthesis" (Rabinowitch 1945, p. 569). Rabinowitch's 'nightmare' exists by a biochemical

pathway he could not then have anticipated, and this is how this was established.

While I was doing biochemical studies on glycolate metabolism, John P. Decker a plant physiologist in Puerto Rico was doing pertinent experiments at the same time. Commercial infrared CO_2 gas analyzers became available in the 1950s, and using this instrument he observed that after tobacco leaves had achieved steady-state rates of photosynthesis if the leaves were suddenly darkened, there was a momentary rapid outburst of CO_2 before steady-state dark respiration occurred. He correctly deduced that when the light is turned off photosynthesis diminishes more quickly than respiration. Since the initial respiration rate shown as a burst was about three times greater than dark respiration and was a residual of a process that went on in the light, he called it 'photorespiration'.

The editor of *Plant Physiology* at the time, Allan Brown, did not believe there could be a respiration in the light different from dark respiration, much less a faster one, so he deleted the word 'photorespiration' whenever Decker used it in his papers. Decker once sent me reprints of these papers showing how they had been altered by the editor. Decker realized this output of CO_2 was large enough to decrease net carbon assimilation appreciably (Decker 1959). He finally got to use 'photorespiration' in a 1959 paper he published in the *Journal of Agriculture University of Puerto Rico* (Decker and Tió 1959). I probably read some of his papers but did not appreciate the relation of his work to my own until several years later.

In 1963, I had a moment of revelation about glycolate metabolism and photorespiration while attending a photosynthesis conference held in Airlie House, Virginia organized by Bessel Kok and André T. Jagendorf. A talk given by Gleb Krotkov, a plant physiologist from Queens University, Ontario, described experiments confirming Decker's earlier results on the post-illumination outburst using soybean and tobacco leaves (C_3 plants) (Krotkov 1963). Then he showed the outburst increased greatly at higher O_2 levels. Although it is not in the printed version of his talk, he also had a slide showing the outburst was absent in maize leaves (C_4 plants). As I listened to his talk, I could hardly contain myself because I realized that the properties of the outburst could be explained entirely by the characteristics of glycolate metabolism. I thought it might also account for the known faster photosynthetic net carbon assimilation by maize, because here the synthesisof glycolate was somehow slowed. Immediately after Krotkov's talk, during a coffee break, I sought him out and related these ideas to him. He said he did not think glycolate could possibly be involved in the outburst. Nevertheless, I returned home pleased and confident that I was working on a key to understanding more about photorespiration.

HPMS, though hardly a perfect biochemical inhibitor, turned out to be useful when used judiciously. When I added HPMS to tobacco leaf disks in light together with $^{14}CO_2$, the specific radioactivity in the carbon atoms of glycolate accumulating was the same as the $^{14}CO_2$ supplied, confirming that it must be an early product of the carboxylation reaction (Zelitch 1965). In other experiments with leaf disks I gave them HPMS to block glycolate oxidation and soon thereafter exposed the disks to $^{14}CO_2$ for a short time. The HPMS had no effect on stomatal opening under the experimental conditions. At 35 °C, the rate of photosynthetic carbon assimilation was increased about 3-fold by HPMS treatment, while there was no effect at 25 °C. The added inhibitor did not increase photosynthesis in maize, and glycolate levels hardly increased at either temperature. These results (Zelitch 1966) suggested that photorespiration was very temperature-dependent and became a greater fraction of gross photosynthesis at higher temperatures in C_3 plants.

Must photorespiration be seen to be believed?

John Decker was not alone in experiencing disbelief about the reality and importance of photorespiration. I recall the public skepticism directed at me by James Bonner, a prominent plant physiologist, when I stated in a talk at the photosynthetic symposium, organized by Anthony San Pietro, called 'Harvesting the Sun' in Chicago in 1966 that photorespiration must substantially reduce net photosynthesis in C_3 plants (Zelitch 1967). "Did you really mean to say that?" he asked derisively. "That is exactly what I mean", I responded. The truth is that I derived secret pleasure in knowing that even well known scientists had difficulty understanding what I confidently thought I understood, namely that an important process like photorespiration had previously been missed. I suppose I was fortunate because I did not have to apply for peer-reviewed grant funds at that time to work on such an 'imaginary' process like photorespiration.

Such skepticism was widespread as was the belief that photorespiration was an artifact. For instance,

in his scholarly book on the physiology of photosynthesis, the distinguished English plant physiologist O.V. S. Heath wrote that it is 'problematical' whether photorespiration occurs at high rates in an atmosphere containing normal levels of CO_2 (Heath 1969). For a discussion of a somewhat similar skepticism in the area of the mechanism of photophosphorylation, see Jagendorf (1998).

What's in a name? The term 'photorespiration' itself was also not widely accepted for some time. In 1969, there was a symposium held on photorespiration at the International Botanical Congress in Seattle. During a discussion period, Hans Gaffron questioned the use of 'photorespiration' and said it was misleading. He argued that even though glycolate was synthesized in the light, its oxidation to CO_2 was a 'dark' reaction. I pointed out that 'photosynthesis' also included the 'dark' reactions by which CO_2 is reduced to carbohydrate and that perhaps we should also throw out the word 'photosynthesis'. 'Photorespiration' has largely remained to describe the cyclic process.

Difficulties in assaying photorespiration and controversy about its stoichiometry

I realized from the beginning that it would not be easy to assay photorespiratory CO_2 release in a quantitative manner because there was too much competition from the main flux of CO_2 being fixed by chloroplasts in the leaf. Photorespiration turned out to be a cyclic process involving a number of biochemical transformations arising from glycolate (Figure 2) in which the CO_2 is released primarily at a later step during the condensation of two glycine molecules to produce serine (Tolbert 1971). It is a futile cycle since there is no net disappearance or accumulation of any metabolite and the only product, CO_2, is recycled and cannot be measured directly. A number of ingenious methods of assay in leaf tissue are described in the literature. All have some advantages and disadvantages. An uncertainty shared by many methods results because they are not done under physiological conditions. And even when the assay is done under normal conditions, almost all of the released CO_2 is recaptured by the faster rate of photosynthesis,hence photorespiration is underestimated to some extent, and this is especially true when photorespiration is low relative to net photosynthesis.

A photorespiration assay that I exploited in the early days had many such limitations, but it revealed useful new information. It was based on the rate of release of recently fixed $^{14}CO_2$ in a rapid stream of CO_2-free air from tobacco leaf disks at 30–35 °C. Rates of photorespiration were 3–5 times greater than dark respiration, while in maize photorespiration was only 2% of that in tobacco. I found that HPMS inhibited photorespiration but not dark respiration, and the photosynthetic inhibitor CMU [3-(*p*-chlorophenyl)-1,1-dimethylurea] acted similarly (Zelitch 1968). These experiments supported the role of glycolate metabolism in photorespiration and showed that it is virtually lacking in C_4 plants.

Glycolate arises from the oxygenase reaction of RuBP carboxylase/oxygenase (Bowes et al. 1971), from phosphoglycolate that comes from carbon numbers 1 and 2 of RuBP. Photorespiration is stimulated by higher O_2 levels, lower CO_2 concentrations, and higher temperatures. These factors also increase the partitioning of the enzyme products to relatively more of the oxygenase reaction. Based on studies with the pure enzyme and models based on the CO_2 compensation point, and taking the value of 77 for the specificity factor for *Nicotiana tabacum* (Ogren 1984) one can calculate the expected carboxylase/oxygenase ratio at 25 °C in 21% O_2 (258 μm). If one assumes that in normal air the CO_2 concentration in the chloroplasts is 300 μl l^{-1} (9.89 μm), the carboxylase/oxygenase ratio is 3:1, and photorespiratory CO_2 will be 20% of net photosynthetic carbon assimilation. The CO_2 concentration reaching chloroplasts in a leaf is less than the atmospheric concentration because of stomatal and mesophyll cell diffusive resistances, and it is also affected by irradiance. At 25 °C in 21% O_2, if the CO_2 concentration in the leaf chloroplasts becomes less than 300 μl l^{-1}, photorespiratory CO_2 will be more than 20% of net photosynthetic carbon assimilation according to this model.

Temperature greatly stimulates photorespiration relative to net photosynthesis, as I had concluded earlier, largely because increasing temperature reduces the affinity of RuBP carboxylase for CO_2 (Ogren 1984). The failure of C_4 plants to synthesize much glycolate results from the high CO_2 levels in the bundle sheath compartment, where RuBP carboxylase is present, causing a suppression of the oxygenase activity (Hatch 1971).

The effect of CO_2 levels, O_2-concentration and temperature on net photosynthesis and photorespiration in tobacco leaf was reported by Richard B. Peterson, a department colleague, who made an important advance in assaying photorespiration quantitatively in intact tissue under physiological conditions.

He developed a computer based model to determine the initial rate of the post-illumination CO_2 burst in an open continuous flow system after steady state CO_2 assimilation was reached (Peterson 1983a). In one set of experiments, for example, he found at 334 μl l^{-1} CO_2 and 21% O_2 the ratios of photorespiration/net photosynthesis were 0.25, 0.45, and 0.90 at 25 °C, 30 °C and 35 °C, respectively (Peterson 1983b).

A novel method of determining photorespiration rates was developed by Kenneth R. Hanson, a department colleague experienced in stereochemcial methods used to investigate enzymatic reaction mechanisms. He supplied a tracer quantity of stereospecifically trititated glycerate to leaf tissue in the light, and it was converted to tritiated glycolate (Figure 2). This became an internal stereochemical probe of the photorespiratory pathway, since the loss of tritium to water occurs only through the action of glycolate oxidase. The magnitude of the loss reflects flux through the pathway (Hanson and Peterson 1985, 1986).

The stoichiometry of photorespiration, the fraction of glycolate-C metabolized to CO_2 as it occurs in the pathway is 25%, because two glycolate molecules are needed to convert two glycines to serine and CO_2 (Figure 2). Since glyoxylate and hydroxypyruvate are compounds in the photorespiratory pathway that are so easily oxidized by H_2O_2 generated by glycolate oxidase, it seemed possible that excess CO_2 might 'leak' from the cycle by peroxidation and thereby increase the stoichiometry above 25%. This would most likely happen at high temperatures, low CO_2 or high O_2 levels when photorespiration increases greatly.

In experiments using the post-illumination burst and the stereochemical probe simultaneously, Hanson and Peterson (1985, 1986) concluded that under conditions of high photorespiration, the fraction of glycolate carbon photorespired was not constant and did increase above 25%. When experiments are conducted using both probes simultaneously, there may be unknown errors in each assay in one direction or another depending on the conditions, so these results may not be the final word. Nevertheless, their results keep open the question of whether the stoichiometry is always constant. This uncertainty has considerable bearing on methods that might be used to decrease photorespiration and increase net photosynthesis, as I will discuss later.

Attempts to decrease photorespiration and increase net photosynthesis

I have always had my eye out for biochemical and genetic changes that might be used to increase net photosynthesis, since plants with this characteristic in the hands of plant breeders could produce crops with increased yields without additional inputs (Zelitch 1982, 1992). Certainly CO_2 enrichment in greenhouses and even outdoors has consistently increased net photosynthesis and yields in C_3 plants.

Since photorespiration seems wasteful, it would seem that decreasing the oxygenase/carboxylase ratio of RuBP carboxylase somewhat would be a logical method of reducing photorespiration and increasing net photosynthesis. This has not yet been accomplished. The attempts to do this and the problems that must be surmounted have been reviewed (Hartman and Harpel 1994; Spreitzer 1999).

However, some biochemists believe that even if it were achieved, it might not be successful in increasing carbon assimilation because the RuBP carboxylase activity is so finely controlled that the maximal rate of carboxylation may not be increased by such a manipulation. This view, without saying so, implies that the evolution of this enzyme has reached its apex and no further improvements are possible. I believe that the long history of plant improvement brought about by conventional plant breeding, and the current miracles being achieved in changing the genetic composition of plants using recombinant DNA methods, argue against the view that evolution cannot be improved upon.

Some time ago I began a search for biochemical inhibitors that would block glycolate synthesis and increase CO_2 assimilation. In a number of experiments glycidate (2,3-epoxypropionate) accomplished this in short term experiments (Zelitch 1974) by some indirect mechanism. Arthur L. Lawyer, a graduate student at Yale who worked in our laboratory, showed that glycidate was a strong inhibitor of the glutamate:glyoxylate aminotransferase reaction (Lawyer and Zelitch 1978), and we thought the temporal increases in net photosynthesis might be caused by a metabolic regulation induced when glutamate or glyoxylate concentration increased. These increases in net photosynthesis, though reproducible, could be sustained for only brief periods and the mechanisms by which this occurred are still not understood.

Photosynthesis in C_3 plants is inhibited 33–55% at 21% O_2 compared to 1–3% O_2, mainly because photorespiration increases with increasing O_2. I car-

ried out a selection for O_2-resistant photosynthesis in tobacco by screening dihaploid plantlets derived from anther culture in an atmosphere of high O_2 and low CO_2 (42% O_2/160 μl l^{-1}) for 2 weeks. Most plants yellowed and some died under these adverse conditions, while a small number remained green and grew. Fertile plants were obtained, and at 30 °C and 21% O_2, net photosynthesis was about 15% greater in the O_2-resistant plants. At 38 °C in normal air net photosynthesis was significantly greater in mutants compared to wild type than at 30 °C (Zelitch 1989).

I suggested that the increased photosynthesis in these mutants was associated with an increased stoichiometry of CO_2 release during photorespiration, because the selected resistant plants had about 40% higher catalase activity on a fresh weight, protein or leaf area basis. In M_4 progeny, about 60% of the plants from the selection had progeny with high catalase activity and the phenotype cosegregated precisely with O_2-resistant photosynthesis that was 25% greater than wild type in 42% O_2 (Zelitch 1990a). The mutant displayed a progressive increase in net photosynthesis relative to wild type with increasing O_2, and the faster rate was completely reversed on returning to 21% O_2 (Zelitch 1990b). Although the matching of catalase activity and resistance represented a perfect correlation, it did not prove a direct relationship. Increases in catalase could have been a consequence of increased photosynthesis rather than its cause. For this reason, I decided to produce plants with cloned catalase genes to test this further.

In collaboration with Neil P. Schultes, Brian McGonigle and Timothy Nelson, we cloned a full-length cDNA from *Nicotiana tabacum* representing the primary leaf catalase (Schultes et al. 1994). Louise Brisson, an energetic postdoctoral colleague from Quebec, joined the laboratory and produced transgenic tobacco plants with a number of different constructs that would either overexpress or underexpress catalase. Evelyn Havir, a colleague in the department skilled in protein chemistry, examined transgenic plants overexpressing catalase and showed that the major leaf catalase protein was in fact increased.

As might be expected, I found that as catalase activity decreased to 20% of wild type there was a significant linear *increase* in the CO_2 compensation point at a leaf temperature of 38 °C (at 50% lower catalase it increased 39%). The increase in the CO_2 compensation point occurs presumably because there is insufficient catalase to completely block peroxidation of ketoacids, and additional CO_2 'leaks' from the cycle. As catalase activity increased up to 90% greater than wild type in transgenic plants, I found a significant linear *decrease* in the CO_2 compensation point at 38 °C (at 50% higher catalase it decreased 17%), but at 29 °C, the trend was not significant. Rates of dark respiration were the same in transgenic and wild type leaves (Brisson et al. 1998).

The results with the oxygen-resistant mutants with elevated catalase and the transgenic plants that overexpress catalase suggest an increased stoichiometry of CO_2 release can be minimized with higher catalase levels when photorespiration is rapid. They suggest a method of regulating photorespiration and enhancing net carbon assimilation by maintaining the stoichiometry of CO_2 formation closer to the minimum 25% per mol of glycolate oxidized. Higher temperatures are often encountered outdoors, and further work must establish whether there is a threshold of temperature before the benefit of greater catalase activity can be observed.

Block Island, Rhode Island

Photosynthesis and the practice of vegetable gardening

Some time ago, quite accidentally, Ruth discovered Block Island to be an attractive, unspoiled place ideal for us to vacation in and become refreshed mentally. It is a small island 10 square miles in size located 13 miles out in the Atlantic Ocean, accessible by ferry from Point Judith, Rhode Island which is about 2 hours from our home on the mainland. For a number of years, we rented houses there for 2 weeks each summer, and in 1982, after we found our family shared our excitement about the Island, we bought a house there. It came with sufficient land and open space for me to do some vegetable gardening and for Ruth to do flower gardening.

My first attempts on a small plot were not very outstanding. I learned that the glacial soil had a pH about 4.5, which could be corrected, that roaming deer would create havoc, and that not all varieties thrived in the maritime environment. Gradually, I started applying what I call 'practical plant physiology' from the starting of seedlings, to optimal spacing, to planning planting times, the use of row covers, and growing adapted varieties. The results got better and better and the small plot grew bigger and bigger.

I thought I would enjoy doing some popular writing about some of my experiences and the relation of

science with vegetable gardening. A magazine called *Kitchen Garden*, later changed to *Kitchen Gardener*, has a section each issue called 'The Science of Gardening', and I have recently published articles in that section (Zelitch 1999a, b, c, 2000). One was called 'Making the sun work for you' and subtitled 'The more you know about photosynthesis, the better your garden can be'.

At the behest of our grandchildren, Ruth and I, and our visiting grandchildren, began selling our produce every Saturday morning at the Block Island Farmer's Market. We enjoy talking to customers about gardening, and people line up and even compete for places to buy our vegetables. Lettuce grows particularly well in that environment, and the 12 varieties that I grow look and taste great because the sandy loam soil and lower temperatures than are found in summer on the mainland are ideal for lettuce. I even became something of a media star last summer when the local weekly newspaper did a story about our garden and bestowed me with the title of 'lettuce king of Block Island'. Now, cold winter nights can find me scrutinizing seed catalogs and planning new things to try out the next summer while pursuing my hobby of practical plant physiology.

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